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A comparison between the use of recombinant hirudin and heparin during hemodialysis

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A comparison between the use of recombinant hirudin and heparin during hemodialysis. The purpose of this study was to determine the anticoagulant and antithrombotic potential of hirudin during hemodialysis by comparing the efficacy of dialysis with heparin to that of dialysis with recombinant hirudin (r-hirudin). Eleven patients with chronic renal failure and on maintenance hemodialysis were included in this open cross-over study. Conventional doses of heparin were administered during the first dialysis of the study. Two days later r-hirudin, at a dose of 0.15 mg/kg, was given as a bolus at the start of the second dialysis. The mean decreases in plasma levels of urea, uric acid and creatinine were approximately 50% after dialysis with both anticoagulants. Dialysis was therefore equally effective. However, effective dialysis with r-hirudin was achieved with a shorter activated partial thromboplastin time (APTT; range 65 to 103 seconds) compared to that with heparin (> 120 seconds), thereby decreasing the risk of bleeding. Markedly less ^{111}In-labeled platelets accumulated at the inlet of the artificial kidney when r-hirudin was used, suggesting a smaller loss of hollow fiber volume. The results indicate that hirudin may be a suitable alternative anticoagulant for use during hemodialysis and it thus warrants further investigation.

Extracorporeal thrombogenesis is a major problem associated with hemodialysis. The composition of the artificial membrane in the extracorporeal system and the large surface area to which the blood is exposed, contribute significantly to activation of the coagulation cascade, white blood cells and blood platelets [1]. The use of an anticoagulant is therefore a prerequisite to prevent thrombotic occlusion of the artificial kidney to ensure effective dialysis. Standard unfractionated heparin is currently the anticoagulant of choice. Its anticoagulant effect is achieved through interaction with the natural thrombin inhibitor, antithrombin III (AT III). Heparin potentiates the inactivation of thrombin by forming a complex with its cofactor AT III, which serves as a template to which thrombin binds [2]. Heparin does, however, not completely prevent thrombogenesis [3], and there are several complications associated with its long-term use. These include thrombocytopenia, increased bleeding tendency, osteoporosis, increased lipolytic activity, and changes of lipid patterns [4–8]. Activation of lipolysis by heparin during hemodialysis also leads to immunosuppressive effects [7]. Therefore a safer and more effective anticoagulant would be beneficial.

Hirudin, the most potent natural inhibitor of thrombin, seems to be a promising alternative anticoagulant for heparin. Hirudin is a direct thrombin inhibitor and does not require endogenous cofactors. It reacts with thrombin in a 1:1 molar ratio to form a noncovalent complex. The carboxy terminal region of hirudin, which is rich in acidic residues, binds ionically to the anion binding exocite of thrombin. The amino-terminal region binds via hydrophobic interaction to the apolar binding site and the Pro 46-Lys 47-Pro 48 region occupies the basic specificity pocket of the active site [9]. Through this mechanism hirudin inhibits all the actions of thrombin and so effectively inhibits coagulation and prevents heparin-resistant arterial-type thrombosis when given in large enough dosages [10]. Hirudin has no adverse effects when infused into humans, because it is pharmacologically virtually inert [11]. Hirudin is also a weak immunogen [12–14]. Hirudin was first used during hemodialysis in 1926 [15]. However, impurities in the crude preparation and the uncertainty about its anticoagulant activity and side effects limited its clinical use. Purified hirudin is now made in sufficient amounts by recombinant technology, and its use during hemodialysis has already been studied in dogs [16] and man [17]. We evaluated the use of recombinant hirudin as an anticoagulant during hemodialysis in humans. We assessed the efficacy of dialysis by comparing r-hirudin with heparin. To achieve this, we measured the biochemical profile, platelet count, hematocrit, platelet function, and coagulation before, during, and after dialysis.

Methods

Subjects

Eleven patients (median age 37 years, range 22 to 51), with chronic renal failure (creatinine clearance < 10 ml/min) and on maintenance hemodialysis were included. The patients gave informed consent to participate. The study was approved by the Ethics Committee of the Provincial Administration and the University of the Orange Free State. The use of r-hirudin was approved by the South African Medicines Control Council. All patients refrained from taking drugs that may influence platelet function and fibrinolysis for at least 10 days prior to, and during the study.

Study design

This was an open cross-over study. Standard unfractionated heparin from porcine intestinal mucosa (Heparin Sodium, Labethica, SA) and r-hirudin (Hoechst AG, Frankfurt and Behringwerke AG, Marburg, Germany) were used. Because of the prolonged elimination of hirudin in terminal renal failure [18],

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Table 1. The mean decrease in serum levels of solutes during dialysis and the percent efficacy of dialysis with heparin and hirudin respectively

	Hirudin		Heparin	
	Decrease	% Efficacy	Decrease	% Efficacy
Urea	13.2 ± 1.9 mmol/liter	50 ± 7	15.7 ± 3.7 mmol/liter	51 ± 7
Uric acid	0.2 ± 0.1 mmol/liter	48 ± 14	0.3 ± 0.1 mmol/liter	50 ± 11
Creatinine	423 ± 152 μmol/liter	45 ± 6	439 ± 149 μmol/liter	44 ± 6

r-hirudin was administered during the second dialysis of the study. This was to avoid possible effects of residual r-hirudin during the second dialysis. Cuprammonium rayon type artificial kidneys (Terumo, Japan) were used once and then discarded. Dialysis was done against an acetate buffer on a Gambro AK 10 artificial kidney machine (Gambro, Sweden). Each dialysis session lasted four hours.

The day before the first dialysis of the study (Day 0), blood (53 ml in 7 ml ACD-A) was collected, the platelets isolated and labeled with ^{111}In-tropolone [19] and reinjected. One day later (Day 1), dialysis with heparin was done. It was administered according to the protocol in use at the Dialysis Unit. The total intra-dialysis heparin dose during the four-hour dialysis session varied between 5,000 and 10,000 IU. Two days later (Day 3), the second dialysis was done with r-hirudin. A single bolus dose of r-hirudin, 0.15 mg/kg dissolved in sterile pyrogen-free water, was given at the start of blood flow through the extracorporeal circuit. The heparin and r-hirudin were infused into the arterial side of the extracorporeal circuit, between the pump and the artificial kidney.

In a dose finding study dosages of 0.08 mg/kg (N = 2) and 0.12 mg/kg (N = 2) r-hirudin was found to be inefficient to prevent occlusion.

Study variables

Platelet accumulation at the inlet of the artificial kidney. The accumulation of ^{111}Indium-labeled platelets was measured with a NaI-scintillation probe. The probe was fixed in a position at the inlet of the dialyzer. The radioactivity counts were expressed as the increase over blood radioactivity (baseline). The latter was measured after the first minute of blood flow through the dialyzer.

Laboratory analyses

Blood was collected at the arterial side of the extracorporeal system. Serum levels of creatinine, urea and uric acid were determined using a Technicon DAX analyzer (Bayer Diagnostics, Basingstoke, UK). The platelet count and haematocrit were determined with a Technicon H*1 blood cell analyzer (Bayer Diagnostics) on blood collected in K_3EDTA. Blood was collected in 3.2% tri-sodium citrate (9:1 vol/vol) for the coagulation and *ex vivo* platelet aggregation studies. The activated partial thromboplastin time (APTT; Actin FS, Dade, Miami, FL, USA) and thrombin time (TT; bovine thrombin; Dade) were measured with a Cobas Fibro semi-automated optical system (Roche, Switzerland) [20]. The fibrinogen levels (derived fibrinogen) were measured with an Automated Coagulation Laboratory instrument (Instrumentation Laboratory, Italy) according to the instructions of the manufacturers [21]. The plasma antithrombin III (AT III) levels were measured by means of a standard chromogenic assay kit (Instrumentation Laboratory) with an Automated Coagulation Laboratory instrument (Instrumentation Laboratory) [22]. *Ex vivo* aggregation of platelets in response to ADP (0.5, 1.0, 1.5, 2.0, 10, and 20 μM) and epinephrine (10 μM) was measured as described in detail [23]. Spontaneous platelet aggregation was also measured. The circulating platelet aggregate ratio (CPAR) was determined as described [24]. The plasma levels of heparin and r-hirudin were determined in blood collected in 3.2% and 3.8% tri-sodium citrate (9:1 vol/vol), respectively. The concentration of heparin in the plasma and dialysate was determined with a chromogenic assay kit (Instrumentation Laboratory) using the Automated Coagulation Laboratory instrument. [25] The plasma and dialysate concentrations of r-hirudin were determined with a chromogenic substrate method [26].

Statistics

The effect of the specific anticoagulant on a variable within a treatment regimen was determined by calculating the mean difference (post-dialysis value − pre-dialysis value), and the 95% confidence interval for the difference. The difference in effect between treatments was determined by subtracting the change during dialysis with r-hirudin from that measured during dialysis with heparin. The 95% confidence interval for this difference was calculated to compare the effect of the anticoagulants during dialysis. The areas under the ^{111}In-labeled platelet accumulation curves were calculated. The mean effects of the anticoagulant, reflected by the relative sizes of the areas, were compared by analysis of variance (ANOVA). The corresponding 95% confidence interval was calculated for the difference between treatments.

A double (hirudin) or single (heparin) exponential function was fitted to the r-hirudin and heparin concentration in time profiles of each individual to determine the plasma distribution and elimination half-lives.

The results are given as a mean ± 1 SD if the results were normally distributed. If not, the median and range are given.

Results

Biochemistry

The mean decrease in urea, uric acid and creatinine after dialysis, and the efficacy of dialysis with heparin and r-hirudin are summarized in Table 1. Dialysis with r-hirudin was as effective as dialysis with heparin since none of the values differed significantly ($P > 0.05$).

Coagulation and plasma levels of heparin and r-hirudin

The changes in activated partial thromboplastin time (APTT) and thrombin time (TT) during dialysis with heparin and r-hirudin, respectively, are summarized in Table 2. Plasma levels of fibrinogen and AT III are given in Table 3. The difference between the decrease in AT III levels during dialysis with heparin,

Table 2. Activated partial thromboplastin time (APTT) and thrombin time (TT) during dialysis with heparin and r-hirudin

	Pre-dialysis	10 min	60 min	120 min	180 min	240 min
APTT, *seconds*						
Hirudin	30 to 38	65 to 103	53 to 82	51 to 75	48 to 76	46 to 70
Heparin	30 to 39	> 120	> 120	58 to > 120	36 to > 120	28 to > 120
TT, *seconds*						
Hirudin	21 to 36	> 180	> 180	> 180	> 180	> 180
Heparin	24 to 38	> 180	> 180	> 180	55 to > 180	35 to > 180

Values are given as a range at each time interval.

Table 3. Changes in fibrinogen and antithrombin III levels during hemodialysis

	Heparin			Hirudin				
	Pre ± SD	[a] MD ± 1 SD	95% CI	Pre ± 1 SD	[a] MD ± 1 SD	95% CI	[b] Hep − Hir ± 1 SD	95% CI
Fibrinogen, *g/liter*	4.0 ± 1.5	0.4 ± 0.7	−0.1; 0.8	3.7 ± 1.2	0.2 ± 0.4	−0.1; 0.5	−0.2 ± 0.6	−0.5; 0.2
AT III, %	83 ± 20	−7 ± 27	−25; 11	84 ± 23	12 ± 23	−3; 29	20 ± 17	8; 31[c]

[a] The effect of the anticoagulant on a variable within a treatment regimen was determined by calculating the mean difference (MD; post-dialysis value minus pre-dialysis value) and the 95% confidence interval (CI).

[b] The difference between treatments was determined by subtracting the change during dialysis with r-hirudin (Hir) from that measured during dialysis with heparin (Hep). The 95% CI for this difference was also calculated to compare the anticoagulants during dialysis.

[c] $P \leq 0.05$

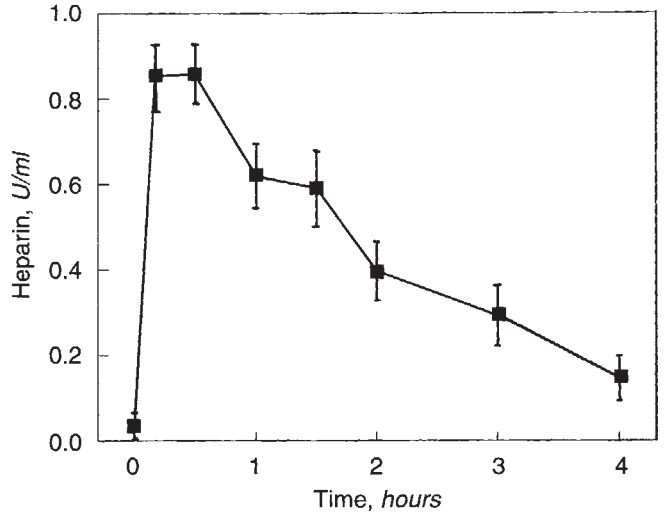

Fig. 1. *Changes in the plasma concentrations of heparin during the four hours of dialysis.* Results are given as a mean ± 1 SEM.

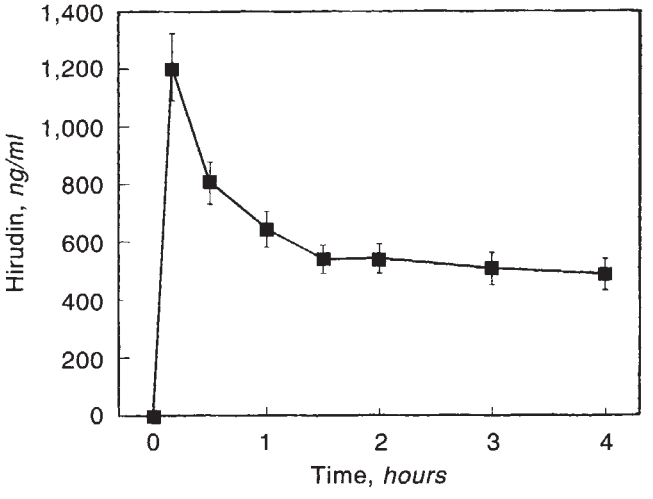

Fig. 2. *Changes in the plasma concentrations of r-hirudin during the four hours of dialysis.* Results are given as a mean ± 1 SEM.

and the increase during dialysis with r-hirudin, was significant ($P < 0.05$).

The changes in the plasma levels of heparin and r-hirudin are summarized in Figures 1 and 2, respectively. The half-life of heparin in the seven patients who did not receive maintenance doses was 66 minutes (median; range from 42 to 225 min). The distribution half-life of r-hirudin was 11 minutes (median; range from 5 to 24 min) and the apparent elimination half-life was nine hours (median; range from 2 to 107 hr). In eight of the patients the elimination half-life ranged from 2 to 12 hours. In the remaining three it was 20, 36 and 107 hours, respectively. No r-hirudin or heparin was measured in the dialysate.

Accumulation of ^{111}In-labeled platelets

The accumulation of ^{111}In-labeled platelets at the inlet of the artificial kidney is given in Figure 3. The median for the areas under the curves (AUC) for r-hirudin (0.66 hr × change from baseline; range −0.22 to 3.88) was smaller than that for heparin (1.22 hr × change from baseline; range −0.36 to 8.09). The 95% confidence interval (CI) for the difference between treatments was from 1.0 to 2.49 hours × change from baseline.

Hematological measurements

Neither the platelet count nor the hematocrit changed significantly within or between treatments.

Platelet function studies

The results are summarized in Table 4. There were no significant differences within or between treatment regimens. However, platelet aggregation in response to the higher concentrations of ADP tended to be slightly inhibited after dialysis with heparin, but not after dialysis with r-hirudin. The finding that there were no

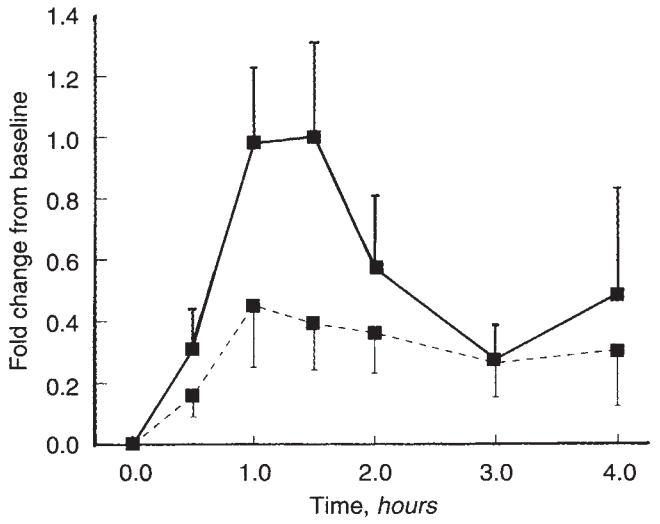

Fig. 3. *The relative accumulation of ^{111}Indium-labeled platelets, expressed as the fold increase from baseline at the inlet of the artificial kidney during dialysis with heparin (–■–) and r-hirudin (- - ■ - -), respectively.* Results are given as a mean ± 1 SEM.

increases in circulating platelet aggregates is surprising, but might have been influenced by the site of sample collection.

Discussion

This study in patients clearly demonstrates that r-hirudin can be used successfully as anticoagulant during hemodialysis. The percent decrease in plasma creatinine, urea and uric acid were equivalent for both anticoagulants, indicating that dialysis with r-hirudin was as effective as dialysis with heparin. When compared to heparin, effective dialysis with r-hirudin was achieved at a shorter APTT (Table 2). This is of particular importance since a prolonged APTT is associated with an increased risk of bleeding [26, 27]. The changes observed in the TT is probably of no clinical relevance because the TT is an extremely sensitive test of thrombin inhibition. Therefore it is not indicative of the anticoagulation status or of a bleeding risk [28, 29].

The longer elimination half-life of r-hirudin is a matter of concern. However, one must take into account that it is perhaps not ideal to derive the long half-lives from the course of plasma levels determined from samples collected only for four hours. Our results does compare favorably with that measured in patients with chronic renal failure and chronic renal disease [18], and is considerably longer than that measured in normal humans and animals [30–34]. The prolonged half-life provides a long-lasting anticoagulant effect and the potential for drug accumulation. This needs to be investigated. The reason for the large variation in elimination times of r-hirudin from the plasma of individual patients is not clear. There was no correlation between the elimination times and the baseline creatinine, urea or uric acid values. The large variation may require that the dosages be individually adjusted when repeated treatments are given. A possible solution may be to treat the dialyzer and not the patient, in which case a dialyzer with a larger cut-off value will have to be used, together with a constant infusion of r-hirudin at the inlet of the dialyzer. In this study the dialyzers had a cut-off value of 5 kD which prevented the removal of r-hirudin by the dialysis process. The elimination half-life of heparin (90 min) was similar to that measured in normal humans [35, 36]. Heparin is partially depolymerized by the cells of the monocyte-macrophage system and the degradation products excreted by the kidneys [36, 37]. The potential for accumulation of degradation products may therefore explain, at least in part, some of the complications of the long-term use of heparin [4–8].

Markedly less ^{111}In-labeled platelets accumulated at the inlet of the artificial kidney during dialysis with r-hirudin. Excessive platelet deposition, especially during the first three hours of dialysis, was prevented by r-hirudin (Fig. 3). This may have resulted in a smaller loss of hollow fiber volume because there is a strong relationship between the number of platelets deposited and loss of volume [38]. In this case dialysis with r-hirudin could have been more effective during this time. Unfortunately we did not measure it. The fact that deposition of platelets after three hours of dialysis were similar for both anticoagulants suggests that a minimum number of platelets will always accumulate in the artificial kidney. This assumption is supported by the findings that D-Phe-Pro-Arg CH_2Cl, a synthetic and direct thrombin inhibitor, completely prevents platelet deposition onto native Dacron vascular graft material [38], but was not able to do the same in artificial kidneys [39].

There are several possible reasons why heparin is not as effective as hirudin in inhibiting arterial thrombus formation. These include: (a) heparin may contain fractions that activate platelets directly, thereby promoting thrombogenesis [40]; (b) activated platelets release proteins, such as platelet factor 4 and beta-thromboglobulin, that can inactivate heparin locally at the surface of thrombi [41]; (c) the inactivation of thrombin bound to other components in a thrombus may be prevented by steric or ionic hindrance of the heparin-AT III complex [42]; and (d) platelets within a thrombus may be activated by meizothrombin which is not inactivated by the heparin-AT III complex [43].

The AT III levels decreased during dialysis with heparin and increased during dialysis with r-hirudin. The decrease after heparin dialysis is not surprising and can be attributed to consumption of AT III by heparin to inactivate thrombin [44]. However, the decrease was not substantial and is possibly of little clinical significance. Moreover, the decrease was transient since the AT III levels before dialysis with r-hirudin were similar to that before dialysis with heparin. The increase in AT III levels after dialysis with r-hirudin is difficult to explain. It cannot result from hemoconcentration [45] since the hematocrit did not change markedly. AT III is an acute phase protein. The slight increase during dialysis with r-hirudin may indicate that there was a slight consumption of AT III. That can be explained by the pre-phase enzyme activation of Factor IX, Factor X, and others. When r-hirudin is used, these factors are activated and bound by AT III.

To our knowledge only one study investigated the use of r-hirudin during hemodialysis in humans [17]. In that study a dose of 0.08 mg/kg was effective to prevent occlusion of the extracorporeal circuit. In our dose finding study this dose and even a higher dose of 0.12 mg/kg was too low to prevent thrombus formation in the extracorporeal circuit. We therefore used a dose of 0.15 mg/kg.

In conclusion, this study demonstrates the feasibility of using r-hirudin in patients undergoing hemodialysis. The results indicate that an intravenous bolus of 0.15 mg/kg r-hirudin was safe and effective. Since it is administered as a single pre-dialysis dose,

Table 4. Changes in the circulating platelet aggregate ratio (CPAR) and platelet aggregation during hemodialysis

	Heparin			Hirudin				
	Pre ± 1 SD	[a] MD ± 1 SD	95% CI	Pre ± 1 SD	[a] MD ± 1 SD	95% CI	[b] Hep − Hir ± 1 SD	95% CI
CPAR	0.81 ± 0.14	0.00 ± 0.11	−0.07 ; 0.07	0.78 ± 0.12	0.05 ± 0.13	−0.04 ; 0.13	0.04 ± 0.19	−0.08 ; 0.17
Aggregation, %								
Spontaneous	4.6 ± 1.6	1.0 ± 1.7	−0.03 ; 2.2	4.5 ± 2.0	0.6 ± 2.7	−1.3 ; 2.5	−0.4 ± 3.5	−2.9 ; 2.2
Adrenalin 10 μM	55.9 ± 9.6	−3.2 ± 10.5	−1.07 ; 4.3	58.9 ± 8.4	−7.3 ± 16.0	−18.7 ; 4.1	−4.1 ± 18.5	−17.3 ; 9.1
ADP : 0.5 μM	9.0 ± 5.6	2.2 ± 3.9	−0.8 ; 5.2	15.9 ± 14.8	−4.5 ± 6.9	−8.6 ; 3.6	−3.8 ± 6.3	−9.1 ; 1.5
1.0 μM	26.9 ± 16.2	−4.5 ± 17.2	−16.8 ; 7.8	25.1 ± 15.7	−4.0 ± 10.1	−11.2 ; 3.2	0.5 ± 23.9	−16.6 ; 17.6
1.5 μM	46.5 ± 16.4	10.0 ± 19.0	−23.7 ; 3.5	44.6 ± 13.5	0.0 ± 19.5	−13.9 ; 13.9	10.1 ± 16.6	−2.0 ; 22.1
2.0 μM	52.6 ± 16.2	−6.9 ± 13.4	−16.5 ; 2.7	52.0 ± 10.8	−1.0 ± 16.9	−13.1 ; 11.1	5.9 ± 20.7	−8.9 ; 20.7
10.0 μM	61.6 ± 9.8	−5.8 ± 9.1	−12.2 ; 0.8	61.6 ± 10.7	1.8 ± 12.1	−6.9 ; 10.5	7.6 ± 15.4	−4.5 ; 17.6
20.0 μM	63.0 ± 9.3	−3.6 ± 8.5	−9.7 ; 2.5	65.4 ± 12.4	2.9 ± 12.1	−5.7 ; 11.6	6.6 ± 15.4	−4.5 ; 17.6

[a] The effect of the anticoagulant on a variable within a treatment regimen was determined by calculating the mean difference (MD; post-dialysis value minus pre-dialysis value) and the 95% confidence interval (CI).

[b] The difference between treatments was determined by subtracting the change during dialysis with r-hirudin (Hir) from that measured during dialysis with heparin (Hep). The 95% CI for this difference was also calculated to compare the anticoagulants during dialysis.

it will simplify the dialysis procedure. The shorter APTT indicates a reduced risk of developing a bleeding tendency. The fact that less platelets accumulated at the inlet of the dialyzer indicates that the dose was sufficient to protect against thrombus formation. In addition no side effects, bleeding complications or thrombotic events occurred with the use of r-hirudin. These findings, together with the fact that r-hirudin can be used successfully in patients with an AT III deficiency and in patients with heparin-induced thrombocytopenia [35], indicate that r-hirudin may be a suitable and better alternative for heparin as anticoagulant. In this study r-hirudin was used in only one dialysis session in each of a relatively small number of patients. Therefore, final conclusions and recommendations cannot be made. It will be worthwhile to investigate the use of r-hirudin in a larger number of patients who are undergoing serial dialysis two to three times per week.

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